CONGLUTINATION IN THE DIAGNOSIS OF DOURINE (TRYPANOSOMIASIS OF THE HORSE) *

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The preparation of the conglutinating system is explained fully by Stranigg in his article on the diagnosis of glanders.[1] He states that conglutination has been used for the diagnosis of glanders, lues, dysentery, and also for the recognition of different plant albumins.

My experience in working out a good system is on the whole the same as Stranigg's. I would add that it is sometimes difficult to get a powerful ox serum, as there are vast differences in the potency of conglutination of different sera. The best titer in my experience so far is 0.1 c.c. with 0.1 c.c. horse serum as complement and 0.1 c.c. of a 5 percent emulsion of sheep blood. It is important to know that in older samples of ox serum a sediment sometimes is formed; such sera give stronger hemolytic than conglutinating action, hence cannot be used. For this same reason, ox serum can not be kept mixed with salt solution. I used well-preserved serum for two and one-half months without noticing a change of the titer.

The complement in horse serum is very sensitive and loses most of its activity after six or seven hours, but it has this advantage, that it always has the same titer, provided the horse used is in a normal condition. It is well to select the best serum of about fifteen, as it is very important to have a reliable complement. Some sera agglutinate very strongly; for example, the serum of a sixteen-year-old mare agglutinated down to 0.07 c.c. The average titer of the horse complement is 0.05 c.c. The best titer I have obtained is 0.02 c.c. Sera which agglutinate so strongly that the serum control (0.1 c.c.) is not negative, of course should not be used.

The emulsion of sheep blood should have the same density always; therefore Stranigg titrates it each time. It is easier and quicker to find the density with the blood-count apparatus. In my experiments, using 0.1 c.c. of a 5 percent emulsion, I found that the density

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1. Arch. f. Wissensch. u. prakt. Tierheilk., 14, 30, p. 166.

could vary between 700,000 and 900,000 corpuscles without affecting the result. The emulsion can be used for seven days.

As antigen, I used pure trypanosomes and an emulsion of the spleen of a rat which had died of trypanosomiasis (tr. equiperdum); but the latter as antigen proved unsatisfactory. For the preparation of the trypanosome emulsion, I inoculated about twenty white rats and bled

TABLE 1
TITRATION OF OX SERUM

Number of Tube	Salt Solution	Fresh Horse Serum as Complement	Inactivated Ox Serum	Sheep Blood Emulsion (5%)
1	0.75	0.1	0.15	0.1
2	0.8	0.1	0.1	0.1
3	0.15	0.1	0.075 = 0.75	0.1
4	0.4	0.1	0.05 = 0.5 Diluted	0.1
5	0.65	0.1	0.025 = 0.25 1:10	0.1
6	0.8	0.1	0.01 = 0.1	0.1
7	0.9	.....	0.1	0.1
8	0.9	0.1	.....	0.1
9	1.0	.....	.....	0.1

Three hours at 37 C.
Twice the smallest amount giving complete conglutination is used as titer.

TABLE 2
TITRATION OF COMPLEMENT

Number of Tube	Salt Solution	Fresh Horse Serum	Inactivated Ox Serum	Suspension of Sheep Blood
1	0.8	0.1	×	0.1
2	.....	0.09 = 0.9	×	0.1
3	0.1	0.08 = 0.8	×	0.1
4	0.2	0.07 = 0.7	×	0.1
5	0.3	0.06 = 0.6	×	0.1
6	0.4	0.05 = 0.5 Diluted 1:10	×	0.1
7	0.5	0.04 = 0.4	×	0.1
8	0.6	0.03 = 0.3	×	0.1
9	0.7	0.02 = 0.2	×	0.1
10	0.8	0.01 = 0.1	×	0.1
11	0.9	0.1	.....	0.1
12	0.9	.....	×	0.1
13	0.85	0.1	×/2	0.1
14	1.0	.....	.....	0.1

The result is read after three hours at 37 C.
The smallest amount of horse serum giving complete conglutination is the titer.

as many of them after two to three days as proved to be heavily infected. The blood was mixed with 1 percent citrate solution and centrifugalized. The trypanosomes, which constitute the superficial layer of the sediment, are then separated with the pipette, the process being repeated as often as necessary. In addition the trypanosomes are thoroughly washed, as it is important to remove all citrate and rat serum,

because these substances hinder the conglutination, even tho only a minute amount is present. The density of the final emulsion may be 1:100. I append the different titration tables. Usually I read the reactions three hours after I add the ox serum and the emulsion of sheep blood. I cannot convince myself that it is an advantage to wait eight hours, as Stranigg does.

The antigen acts similarly as in the usual complement-fixation; the presence of the horse serum decreases the anti-complementary action of the antigen even to a higher degree. Therefore, it is best always to titrate it with the positive and negative serum. One can use the antigen about two weeks, if it is clean, and it is sufficient to titrate it every third day.

TABLE 3

TITRATION OF ANTIGEN

Number of Tube	Salt Solution	Complement	Serum	Antigen
1	To make 1 c.c.	As determined by titration.	Dourine S. 0.1	0.02
2			Dourine S. 0.1	0.05
3			Dourine S. 0.1	0.1
4			Dourine S. 0.1	0.15
5			Dourine S. 0.1	0.2
6			Dourine S. 0.1	0.25
7			Dourine S. 0.1	0.3
8			Dourine S. 0.1	0.35
9			Dourine S. 0.1	0.4
10			Dourine S. 0.1	.....
11			Normal S. 0.1	0.1
12			Normal S. 0.1	0.2
13			Normal S. 0.1	0.3
14			Normal S. 0.1	0.4
15			Normal S. 0.1	.....

After one hour at 37 C. add ox serum and emulsion of sheep blood.
The result is read after three hours at 37 C.

The test serum must be absolutely clean; chemicals and decomposition prevent a satisfactory result and a serum control is always necessary. I inactivate the sera thirty minutes at 59 C.

The reading of the tests, whether conglutinated or not, is very easy, if one has a good system. In the negative tubes, the blood corpuscles appear clumped; in the positive ones, no trace of conglutination is visible.

I have made experiments with nineteen dourine sera, which I hereby tabulate. With the one exception, the results are the same as with complement-fixation, and in some cases, the same as with agglutination.

Two of the thirty normal sera which I tested gave a doubtful result. One of them gave a positive reaction four times, the serum control

showing a partial imbibition. Complement-fixation repeated several times gave absolutely negative results. The agglutination test was doubtful. One other serum gave the same trouble, with the exception that the serum control was not questionable and an agglutination test was not made. Both sera were sent to the laboratory so that clinical observation was not possible.

In the beginning of the experiments, I sometimes had positive reactions in the negative row of the antigen titer; it could be shown that this was caused by contaminated antigen. Preserved antigen and sera proved to be unsatisfactory.

One serum of a donkey gave a negative result, the same as the agglutination. Using the complement-fixation method, one regularly gets positive results with donkey sera, which are therefore very unsatisfactory for use. The donkey mentioned was under observation for a long period and ostensibly healthy.

TABULATION OF THE SERA TESTED

Positive Sera

(1) Stallion, three years old, gave positive complement-fixation, July, 1914. At the same time plaques on the right flank; in one of these plaques, trypanosomes were found; good condition. Used as positive control.

(2) Mare, gave positive complement-fixation since February, 1914. Depigmentation on the vulva; plaques on the right flank; mediocre condition. Used as positive control.

(3) Mare, gave positive complement-fixation since February, 1914. Good condition; no clinical symptoms.

(4) Mare, gave positive complement-fixation since 1913. Mediocre condition; weak in the posterior extremities.

(5) Mare, gave positive complement-fixation until March, 1914; in August of the same year gave only conglutination positive, not complement-fixation. Had been infected by cohabitation in September, 1907; in December of the same year, facial paralysis of the left side; complete recovery after atoxyl treatment.

(6) Mare, gave positive complement-fixation in March and August, 1914. Natural infection March, 1909; no clinical symptoms; good condition.

(7-19) Sera of naturally infected horses, without clinical symptoms, gave equally positive complement-fixation and conglutination.

Negative Sera

(1) Gelding, under observation since birth, was used as negative control.

(2) Donkey, seven years old, gave positive complement-fixation. When the serum was treated with 5 percent carbolic acid, it reacted negatively. The untreated serum, however, gave a positive reaction, when pure trypanosomes were used as antigen, or even a normal rat spleen. The donkey was under observation and showed no clinical symptoms. Negative conglutination.

(3-11) Sera of healthy horses under observation.

(12-21) Sera sent in for test. Among these are the ones mentioned as giving doubtful results. One, the serum of a mule, gave positive complement-fixation; negative conglutination. In no case was it possible to make clinical observations.

(22-30) Nine mares, doubtfully infected or ostensibly recovered, gave negative serum reactions.

CONCLUSIONS

The conglutination method can be used for the diagnosis of dourine; but it is more sensitive to faulty technic and hence more difficult to employ than the usual complement-fixation method.